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Anaerobic and Aerobic Degradation of Aromatic Hydrocarbons Using In-situ Bioreactors at an Unleaded Gasoline Spill Site

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ABSTRACT

Unleaded fuel has leaked from an underground storage tank and spread 100 M radially in the saturated and unsaturated zone under a clay cap of soil. The estimated amount of fuel spilled in the saturated zone is 10,000 L. Four bioreactors have been installed in the ground at the site, one dedicated to anaerobic and three to separate anaerobic conditions: methanogenic, nitrate-reducing and sulfate-reducing. The bioreactors are stainless steel cylinders filled with contaminated zone soil and contain approximately 84 L. Contaminated groundwater is pumped through each bioreactor in an upward flow. Influent and effluent levels of the aromatic hydrocarbons benzene, toluene, ethylbenzene, 1,2,4- and 1,3,5-trimethylbenzene, and m/o/p-xylene were examined. Initially in the aerobic bioreactor using hydrogen peroxide as an oxygen source, only benzene, toluene and the m/o/p-xylenes were degraded to more than 90% of influent concentrations. After conditions were adjusted, the microbial consortia degraded all these gasoline components to more than 90% of influent concentrations. In addition, preliminary studies have suggested anaerobic degradation of these aromatic hydrocarbons, although the biochemical regime remains to be ascertained.

INTRODUCTION AND BACKGROUND

One of the most promising and economical strategies for the destruction of many kinds of organic contaminants is in-situ biotransformation. The objective of this study is to investigate in-situ aerobic and anaerobic degradation of aromatic gasoline hydrocarbons by native groundwater microorganisms obtained from Naval Weapons Station Seal Beach. Aerobic degradation of fuel hydrocarbons by microorganisms is well known. At the Seal Beach site, specific information about aerobic degradation by native organisms was obtained. Unique to this investigation, however, is the anaerobic study. As recently as 10 years ago, the scientific community considered that anaerobic degradation of aromatic hydrocarbons did not occur. Since then, through research conducted at Stanford University and elsewhere, it has been shown that natural microorganisms exist that can degrade aromatic hydrocarbons in the absence of oxygen using nitrate, sulfate and CO_2 as the terminal electron acceptor (Grbic-Galic and Vogel 1987, Ball et al. 1991, Edwards et al. 1991, Haag et al. 1991, Reinhard et al 1991, Beller et al. 1992, Edwards et al. 1992). Such degradation of aromatic fuel components is very promising as a potential remediation strategy, since fuel spills often occur in or create anaerobic conditions in the subsurface.

The components of gasoline that are relatively soluble in water and commonly found as ground water contaminants are the aromatic compounds benzene, toluene, ethylbenzene, and xylenes (BTEX). These compounds are readily degraded by bacteria when oxygen is present as an electron acceptor together with other inorganic nutrients required for bacterial growth. When oxygen is absent or deficient in ground water, these water-soluble aromatics are more persistent. Since organisms exist in the groundwater which utilize alternate electron acceptors (oxidants) such as nitrate, sulfate, or carbon dioxide, studying anaerobic bacterial degradation of BTEX is worthwhile.

Anaerobic laboratory and field studies are generally complicated by the slow rate of organism growth and adaptation to the aromatic hydrocarbons. Although slow, such transformation processes can be significant because of the long residence times of ground water, and therefore are worth studying. Acceleration of these natural processes could come from engineered approaches. A better understanding of the conditions which promote natural degradation would free financial resources and personnel. Better scientific understanding is

needed to know the proper conditions for the microorganisms involved to do their work naturally or with engineering assistance.

SITE AND BIOREACTOR DESCRIPTION

The site description and gasoline contamination information was obtained from a report by the United States Geological Survey (Schroeder 1991), which covers the initial site survey for the extent of the unleaded gasoline plume. Thirty-three wells were placed in the area in 1984-5 (Figure 1). Analysis of soil cores from these wells outlined the plume, which was found to have spread almost radially through the shallow subsoil a distance of 50 to 100 meters from the source. An area of 18,000 square meters and a vertical zone 0.3 to 0.5 meters thick above the shallow water table was contaminated. Seasonal and tidal fluctuations in ground water have spread the gasoline vertically and reduced concentrations below residual saturation over nearly all the area. Total quantity contained in unsaturated subsoils is estimated to be 10,000 liters. The amount present in saturated soils was not determined, but was probably much less.

Only a small hydraulic gradient was expected in the study area. The nearly circular pattern of dispersion for the spreading gasoline reflected this. Tidal influence makes determination of the magnitude and direction of the hydraulic gradient imprecise. These tidal fluctuations have vertically spread nutrients, water and oxygen. Biodegradation is probably enhanced by this fluctuation and may be expected to be more rapid than at most locations where water levels change slowly.

Material in the area is mostly homogeneous sand with silt lenses, with some clay near the surface. Soil in the top 0.3-0.5 meters above the water table is very compact due to the irregular seasonal inundation by tides, and acts as seal against vertical leakage of free product to the surface. The gasoline contamination poses no threat to drinking water supplies. Concern focuses on a nearby tidal marsh, but the concern is mitigated by the fact that free product does not surface because of the clay barrier.

Laboratory information obtained by researchers at Stanford University and Orange County Water District during the first half of this three year project was applied to four bioreactors that were installed at the site in the summer of 1990. The bioreactors, fabricated at the Orange County Water District, consisted of 12-inch diameter by 4 ft lengths of stainless steel pipe sealed at one end and covered by a rounded cap at the other. These were designed to operate in an upflow mode with an influent line at the sealed end of the reactor (Figure 2).

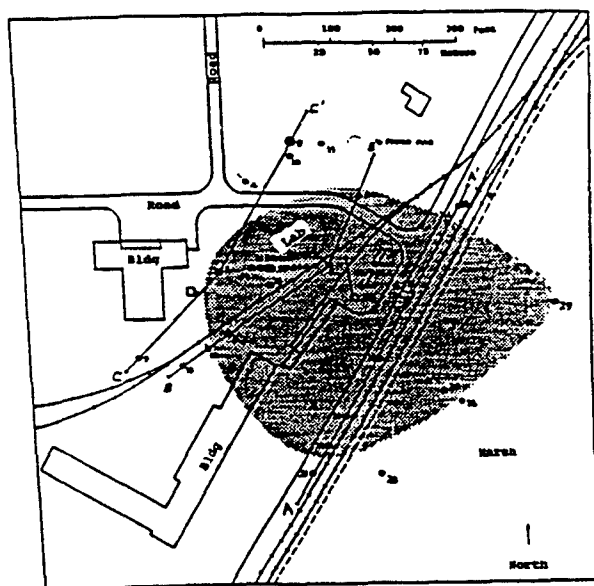


Figure 1
Diagram of Plume

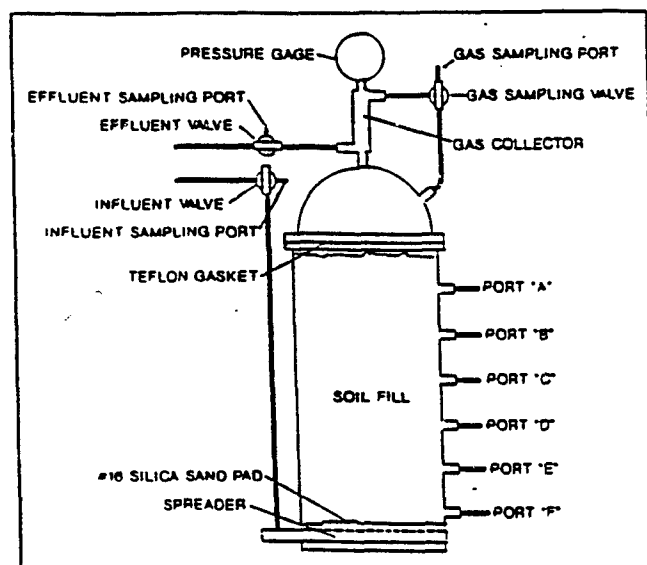


Figure 2
Schematic of bioreactor

Prior to installation, the internal parts of each reactor were sanitized by steam cleaning to help exclude surface microorganisms, and the cap was set in place to maintain sanitary conditions prior to the filling operation. Each bioreactor was placed in an auger-drilled hole 2 ft in diameter by 3.5 feet in depth, and filled with soil excavated from between 8 to 12 feet below the soil surface by a steam-sterilized 2 ft diameter bucket auger. In-ground placement allowed for continuous feed from the contaminated ground water and for the control of temperature by field conditions. To prevent solar heating of the cap, which was flush with the surface, each bioreactor was covered with rectangular 3 x 3 foot x 3.8 inch plywood enclosure which was weatherized and painted white to reflect solar radiation.

The Orange County Water District placed their 30-foot mobile laboratory immediately adjacent to the bioreactors. It housed the pumps, specialized equipment and a preparation room where nutrient solutions were made. Contaminated groundwater from one of the site survey wells, drilled in 1984-85, was pumped through each bioreactor. One bioreactor was operated under aerobic conditions (SBI), the three other bioreactors under anaerobic conditions (SBII, SBIII, and SBIV). Further details of installation and design are in a report by Phipps and Ridgway (1991).

METHODS AND MATERIALS

Laboratory studies with anaerobic batch cultures: Aquifer material was obtained from the Seal Beach site by the Orange County Water District for the anaerobic studies at Stanford University. Either glass columns 40 cm in length and 2 cm in i.d. and narrowed at the bottom (Haag et al. 1991) or glass bottles for batch culturing (Ball et al. 1991, Beller et al. 1992, Edwards et al. 1992) were used. All work was conducted under anaerobic conditions with specified media for the incubation of anaerobic microorganisms.

Field study on the anaerobic bioreactors: Three in-situ bioreactors were installed in June 1990, labelled Seal Beach Two, Three and Four (SBII, SBIII and SBIV). The influent and effluent tubing was changed from Teflon to stainless steel in late July 1990, to prevent any oxygen diffusion through the tubing. These bioreactors can be operated in the closed-loop mode, but will mainly be operated in the flow-through mode (See Figure 2).

Initial characterization of the reactors included (1) baseline studies to determine the level of hydrocarbon contamination and removal, and (2) an experiment for bromide breakthrough to determine void volume and the dispersion coefficient. Wide fluctuations in the contaminant level in the influent line due to tidal fluctuations led to the installation of activated carbon units to act as ballast for the hydrocarbon contamination. These carbon units absorbed hydrocarbons. When the influent groundwater HC contamination dropped, the carbon would release some of its contamination. This helped to level out the baseline.

Nutrient studies were slated to begin in the spring of 1991, but due to problems with ground water pumping systems, and the variation in the contamination level, the nutrient studies were not begun until February 1992.

Laboratory studies on the aerobic soil column: An aerobic column system was used to investigate the gasoline mineralization efficiency of a consortium of organisms obtained from a water sample taken from site well #30. The water sample was aseptically aspirated and allowed to flow through the column, a water-jacketed glass cylinder 2.5 cm diameter x 40 cm filled with #16 silica sand. The nutrient regimen was NPS/5 (Phipps and Ridgway 1991). Unleaded gasoline was fed to the column as its sole carbon source at approximately 1/10 aqueous saturation level. Evolution of CO₂ was continuously monitored as an index of microbial hydrocarbon mineralization with a computerized non-dispersive infra-red gas analyzer system (CNDIR) connected to an IBM clone computer. The column influent and effluent concentrations of benzene, toluene and ethyl benzene were monitored by quantifying these hydrocarbons in discrete samples with a gas chromatograph (GC). Biomass produced by the column was also monitored but not

reported here (Phipps and Ridgway (1991) and Ridgway (1990)).

Field study on the in-situ aerobic bioreactor: The first in-situ bioreactor, installed in early 1990, was labeled Seal Beach One (SBI). Initial operation of SBI was without inorganic supplementation to establish background conditions. Groundwater from well SB 34 was used initially to supply the reactor, but the concentration of hydrocarbons in this well proved to be too variable. The reactor feed was then switched to well SB 2.

Parameters were monitored included physical parameters (temperature and pressure), bacterial counts (total counts and gasoline degraders), BTEX, carbon dioxide, total organic carbon and inorganics (Phipps and Ridgway 1991). Nitrogen, phosphorus and sulfur in NPS/5 media were introduced to the bioreactor and effluent concentrations of the inorganic nutrients monitored. Sulfate broke through immediately, indicating that neither did it exchange with the reactor fill nor did the reactor contain appreciable dead zones. In contrast, phosphate was retarded, and ammonium was most heavily retarded, remaining below influent concentration for more than 30 pore volumes.

Experiments were initiated on 5/1/90 and terminated 233 days later, with a total of nine experiments tested nutrient feeds, hydrogen peroxide addition at different concentrations, removal of hydrogen peroxide and change in nutrient mixes.

RESULTS AND DISCUSSION

Anaerobic laboratory studies: Seal Beach sediments have been shown to biologically mineralize aromatic hydrocarbons without oxygen. In the first of four research efforts described here, tentative evidence for sulfate as a terminal electron acceptor was shown by means of soil columns originally established to develop methanogenic conditions. However, after it became apparent that aromatic hydrocarbon oxidation occurred in the absence of methanogenic conditions, sulfate reducing conditions were studied. In the first of three columns, toluene mineralization was observed, and based on the fact that more than 60% toluene-carbon was converted to CO_2 and that no significant amounts of methane were formed, methanogenesis was excluded as the predominant transformation pathway. No electron acceptor was added, but sulfate was naturally present in the sediment. In the second column, the cumulative removal of toluene approximated the expected stoichiometric ratio if sulfate were the terminal electron acceptor. Addition of sulfate appeared to promote toluene degradation. Toluene was the preferred substrate in the second and third columns, and its presence seemed to slow or inhibit the degradation of other tested materials. In one column, the addition of toluene was discontinued after 126 d, which coincided with the onset of p-xylene degradation (Haag, et al. 1991).

In the second related study, Ball et al. (1991) characterized microbial consortia capable of transforming aromatic hydrocarbons under nitrate-reducing conditions. Batch bottle microcosms under anaerobic conditions were used that had a mixture of aromatic hydrocarbons added to each microcosm. In the first experiment, toluene and phenol were degraded after 222 days of incubation. In the second experiment, microorganisms in an oil refinery sediment (rather than Seal Beach sediment) biotransformed toluene, ethylbenzene and m-xylene with nitrate as the electron acceptor. Inocula from sewage sludges did not adapt to the aromatic compounds tested under denitrifying conditions. Hence, microorganisms with the ability to degrade aromatic hydrocarbons are not ubiquitous, but seem to occur where organisms have been exposed to aromatic compounds for a long time. Toluene was a preferred substrate for biotransformation with different conditions and media tested. Nitrate utilization results gave evidence that biodegradation of ethylbenzene takes place under denitrifying conditions.

Toluene and the three isomers of xylene were completely mineralized to CO_2 and biomass by aquifer-derived microorganisms under strictly anaerobic conditions

in the third study (Edwards et al. 1992). Evidence confirming sulfate as the terminal electron acceptor was shown. Benzene and ethylbenzene were not degraded under the experimental conditions used. Greater than 90 percent of ^{14}C -labelled toluene or ^{14}C -labelled o-xylene was mineralized to $^{14}\text{CO}_2$. The accumulation of sulfide in the cultures as a result of sulfate reduction appeared to inhibit degradation of aromatic hydrocarbons. This biodegradation was shown to be sequential with respect to aromatics, with toluene first, then p-xylene and then o-xylene. The overall biodegradation rate could not be predicted. In a separate experiment, when media with molybdate, an analog to sulfate, was added, sulfate reduction was inhibited and toluene degradation stopped, indicating that sulfate was the electron acceptor (Edwards et al. 1992). A further interesting observation was that benzene was found to biodegrade after about 100 days of acclimation with the microorganisms (Elizabeth Edwards, Stanford University, personal communication).

Beller et al. (1992) found that, in iron-amended microcosms and enrichments, ferric iron reduction proceeded concurrently with toluene degradation and sulfate reduction. The sediment used in this study was from an aviation fuel storage facility near the Patuxent River (MD). Several lines of evidence suggest that toluene degradation was directly coupled to sulfate reduction: (i) the two processes were synchronous and highly correlated, (ii) the observed stoichiometric ratios of sulfate consumed/toluene consumed were consistent with the theoretical ratio for the oxidation of toluene to CO_2 coupled with the reduction of sulfate to hydrogen sulfide, and (iii) toluene degradation ceased when sulfate was depleted, and conversely, sulfate reduction ceased when toluene was depleted. The addition of millimolar concentrations of amorphous $\text{Fe}(\text{OH})_3$ to Patuxent River microcosms and enrichments either greatly facilitated the onset of toluene degradation or accelerated the rate once degradation had begun. The data collected in this study have been used to indicate the importance of iron in either initiating or accelerating toluene degradation and to provide support for a proposed mechanism of iron reduction; however, the data are not suitable for explaining how iron stimulated toluene degradation. Further study will be required to address this issue.

Tidal fluctuation effect: One difficulty at this site were the tidal fluctuations influencing the concentrations of some of the contaminants in the incoming groundwater. Figure 3 plots the concentration of benzene and p/m-xylene versus tide levels. Benzene concentrations were significantly affected by the tides, as were toluene and ethylbenzene (data not shown). The concentration of p/m-xylene remained constant, as did the concentrations of o-xylene and 1,2,4- and 1,3,5-trimethylbenzene (data not shown). Figure 3 demonstrates only the cyclical effect of the tides. Tidal data is from Long Beach Harbor which is 7-8 miles north, and the lag time for the tide to affect the aquifer is not known.

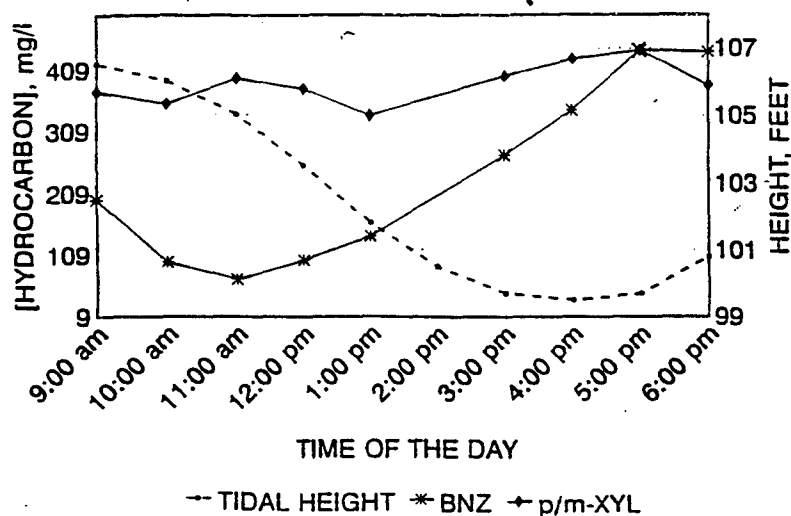


Figure 3

Fluctuations in hydrocarbon concentration in Seal Beach well #35 on 24 Sept 1991

Anaerobic field bioreactors: Figure 4 shows the baseline concentrations of benzene, toluene and nitrate over a two-month period (days 200-260) for four sampling ports (Refer to Figure 2): "Line" is direct groundwater, "influent" after the water has passed through the pumps and the carbon filter (nutrients can be added but have not been in this case), "Port F" is the first sampling port on the side of the bioreactor, and "effluent" is the water coming off the top of the bioreactor. The carbon ballast units were added prior on day 0, and the difference between the influent concentrations in the "Line" and the "influent" for benzene, toluene and nitrate show that the carbon ballast is, in fact, moderating variability in the concentrations of the organics. The data for each of the three bioreactors is similar, which means the bioreactors can serve as replicates of one another.

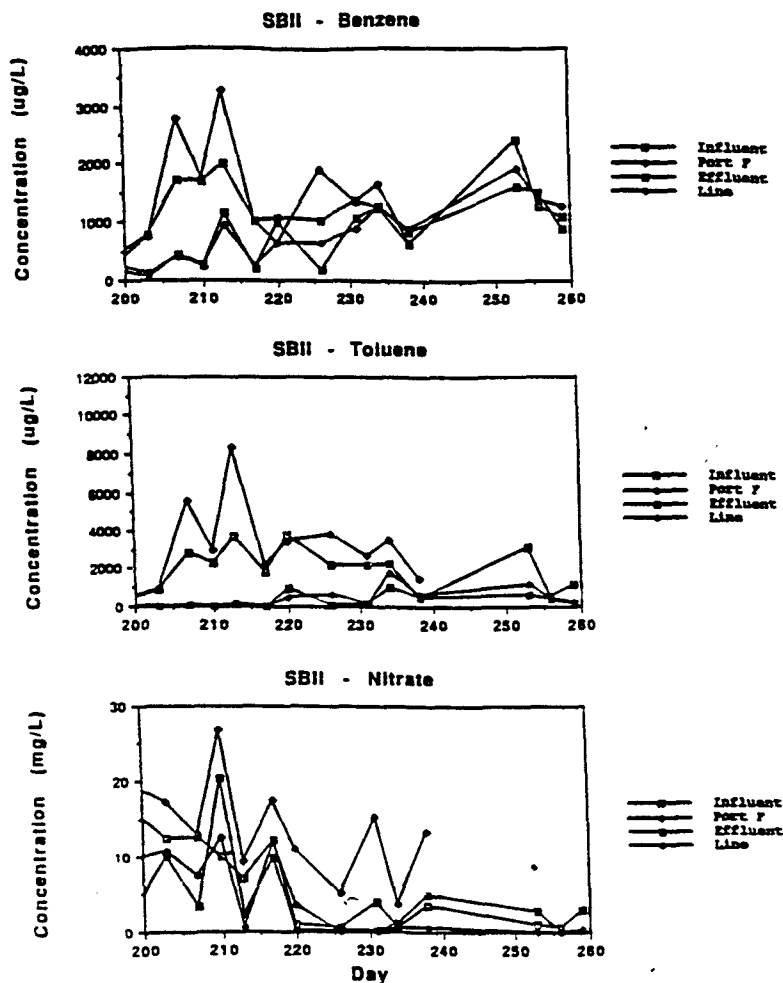


Figure 4

Three aromatic hydrocarbon concentrations from the line feed, influent, effluent, and Port F on bioreactor SBII

At day 220 of this experiment, the nitrate appeared to be reduced and toluene was no longer completely removed. Nitrate may be the limiting factor in this removal. However, the residence time of ground water in the reactor was only nine hours. If the nitrate was exhausted, sulfate could act as the next electron acceptor (Haag 1991) but presumably, longer adaptation and residence times would be required. This question is currently being investigated. Sulfate concentration was 60-80 mg/L in the ground water influent and effluent (Don Phipps, Orange County Water District, personal communication) so the system apparently did not use sulfate before nitrate. The concentration of nitrate was about 15 mg/L in the line feed and was substantially reduced in the reactor effluent, (and even when it acts as the electron acceptor, some nitrate remained).

The three anaerobic bioreactors were shut down for three months in the summer of 1990. In October of 1990, the headspace of SBIII was sampled and analyzed for carbon dioxide and methane. The headspace was found to contain 50% methane and 13% carbon dioxide with the balance presumed to be nitrogen. This was a surprising result as methanogens were not expected to occur. Methanogens may have also been deactivated when the bioreactor was filled by being briefly exposed to oxygen in the air, but apparently these organisms survived the soil transfer. If the methane was a product of hydrocarbon degradation, this would be significant as little is known about methanogenic degradation of hydrocarbons in such aquifer systems. However, it is possible that the methane may have originated in the influent. In October, well head gasses were tested and significant methane and carbon dioxide concentrations were found in several of the wellheads over the contaminated zone and not in those over the uncontaminated zone. Potential methanogenic degradation of hydrocarbons in the contaminated zone merits further study.

Aerobic laboratory data and field bioreactor: Results from the aerobic bioreactor were consistent with the laboratory column findings (Phipps and Ridgway 1991). Although nutrients were added to the field bioreactor, phosphate addition made no difference to biomass or aromatic hydrocarbon degradation. Once the biomass was established, the organisms apparently do not need added nitrogen or phosphorus. The biomass in SBI (aerobic) was sustained 4-5 weeks after removing phosphate. Phosphate exists in the soil at the site and appeared to be at a sufficient concentration for both aerobic and anaerobic biomass. Also, the aerobic biodegradation was complete within the first six to eight inches of soil in the bottom of the reactor. Following introduction of media and 0.005% (1.49mM) hydrogen peroxide, the bioreactor began removing toluene, benzene, and o-xylene. Removal of p/m-xylene might have been taking place before nutrient/peroxide addition, using microaerophilic conditions present in the reactor. Initially, 1,2,4 trimethylbenzene was poorly removed by the reactor, and ethyl benzene behavior was anomalous. Later, both of these were biodegraded to more than 90% of influent concentrations. Figure 5 shows data from experiment 6. The reactor was supplemented with nutrients and hydrogen peroxide, and Figure 5 compares the influent and effluent concentrations of aromatic hydrocarbons. The California maximum concentration levels are added for interest.

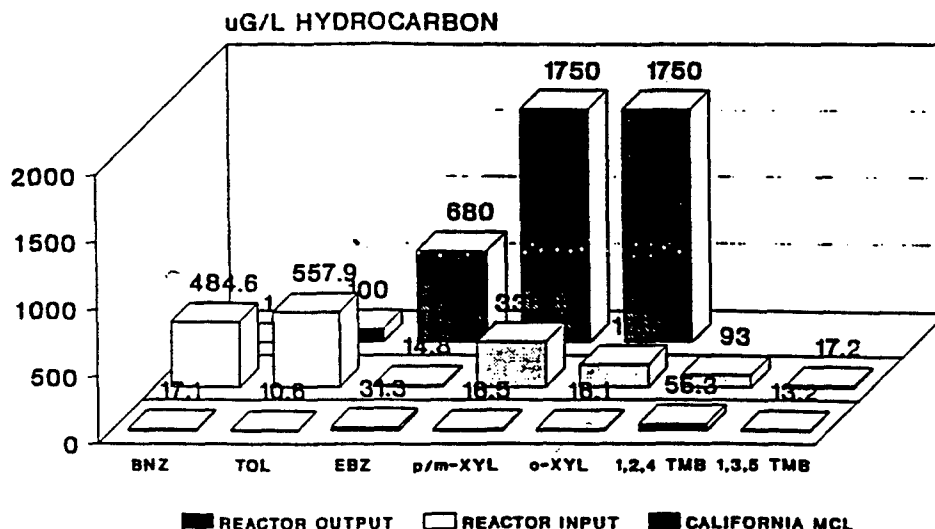


Figure 5

Comparison of BTEX input and output
concentrations to
California Maximum Concentration Levels (MCL)

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